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THE LOCATION OF OLFACTORY RECEPTOR SITES

INFERENCES FROM LATENCY MEASUREMENTS

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ABSTRACT Excitatory responses recorded from vertebrate olfactory sensory neurons are characterized by long latencies compared with those from other sensory receptors. Explanations which assume free access of the stimuli to receptor molecules presumably located on the olfactory cilia necessarily imply an intrinsic delay in the transduction mechanism. In contrast, the possibility of restricted or delayed access due to diffusion of the stimulus to molecular receptors located on the dendritic knob or proximal portions of the cilia suggests transduction processes having time courses similar to those in other sensory systems. We show that the threshold stimulus concentration and the latency of the excitatory response of the salamander can be predicted primarily on the basis of a diffusional delay and that the receptor molecules are well below the surface of the mucus. Examination of response latencies for other species reported in the literature support the generality of diffusional delay. The predicted location of molecular receptor sites is largely insensitive to assumptions based on the mode of clearance of the stimuli. Additional access restrictions are discussed but are shown to generate qualitatively different latency functions than does diffusion, suggesting that they exert only minor influences on latency and threshold characteristics.

INTRODUCTION

The interaction of low molecular weight organic compounds with receptor sites on olfactory receptor cell membranes is the initial step in the transduction process leading to odor recognition. Subsequent neural processing leads to the perception of odor quality and intensity. The vertebrate olfactory receptor cell is a bipolar neuron consisting of a dendrite, soma, and axon which lie in the sensory epithelium lining certain regions of the nasal cavity. Several cilia, up to 200 μm long, extend from the apical knob of the dendrite into the mucus which bathes the epithelial surface. The average thickness of this mucus in frogs is 30 μm (Reese, 1965; Holley and MacLeod, 1977). This suggests that the distal portions of the cilia lie parallel to the surface of the mucus for most of their length. The receptor cell axons project to the olfactory bulb without axon collaterals or synapses. There is considerable controversy in the literature regarding the location of the receptor molecules in the sensory epithelium. It was suggested that the receptor sites are located on the ciliary membrane of the receptor cells (Vinnikov and Titova, 1949; Ottoson, 1956). However, subsequent attempts to demonstrate electrophysiologically that cilia play an essential role in recognition and transduction have been equivocal (Shibuya, 1969; Shibuya and Tucker, 1967; Bronshtein and Minor, 1977). It is

even conceivable that the sustentacular cells have receptor sites and indirectly contribute to the transduction by receptor cells via secretion.

The controversy and speculation surrounding the location of the receptor sites is clouded in a more general problem regarding the role played by the slow transepithelial voltage change recorded from the epithelial surface in response to an odor, referred to as the electroolfactogram (Ottoson, 1956). Although it is typically monophasic negative, it may be multiphasic or biphasic using certain stimuli or under certain recording conditions. The controversy surrounding the interpretation of the cellular sources which generate the slow voltage transient is similar to that surrounding the interpretation of the electroretinogram recorded from the surface of the retina. Because of the ambiguity surrounding the interpretation of the electroolfactogram (discussed in Getchell, 1974; Holley and MacLeod, 1977) we have focussed our study on the unitary action potentials recorded from olfactory receptor neurons. Based upon an analysis of unitary spikes recorded extracellularly as well as intracellular recordings from olfactory receptor neurons, Getchell (1973; 1977a; 1977b) formulated a model which describes the sequential neural steps in the activation of the receptor cell. In addition, a qualitative model developed for heuristic purposes outlines the major perireceptor events that occur in mucus and control access of odor molecules to the receptor sites (Getchell and Getchell, 1977).

Excitatory responses may be recorded from single receptor neurons upon odor stimulation. There is a systematic decrease in the response latency with increasing odor concentration (Getchell and Shepherd, 1978a). In general, the latencies are longer than would be expected from neural membrane events. This suggests that perireceptor events may contribute significantly to the interval between the arrival of the odor pulse at the mucus surface and the initial spike in the excitatory response. In this paper we show that mass transfer processes can account for the extended latencies and their concentration dependence in a quantitative manner.

The importance of physicochemical preneural events in determining the primary olfactory response was initially stressed by Tucker (1963). He concluded that responses to a given odorant depend critically upon the concentration and on the flow rate of the gas conveying it to the epithelium. Reasoning from a diffusion model he showed that at sufficiently high flow rates the microenvironment of the receptors probably reaches equilibrium with the stimulating gas stream. Bostock (1974) presented a diffusion model attempting to account for the time course of the electroolfactogram. He divided the stimulatory process into a series of physicochemical events, including diffusion and partitioning of the odorant. More recently, van Drongelen et al. (1980) further elaborated a diffusion model for the primary events in olfaction. Fig. 1 summarizes some of the physical processes involved in activation of the olfactory receptor neuron as we shall consider them in this communication.

It is our intention to show that diffusion of the odorant through the mucus layer is rate limiting and can account for the observed response latencies when it is assumed that the response requires a certain minimum (threshold) concentration of odorant to be present at the locus of the receptor sites. Analyses of data relating concentrations to response latencies in terms of diffusion limited events permits the estimation of both the apparent threshold concentration and the depth in the mucus where the receptor molecules are most probably located. Our treatment predicts that receptor loci for odorants are well below the gas/mucus

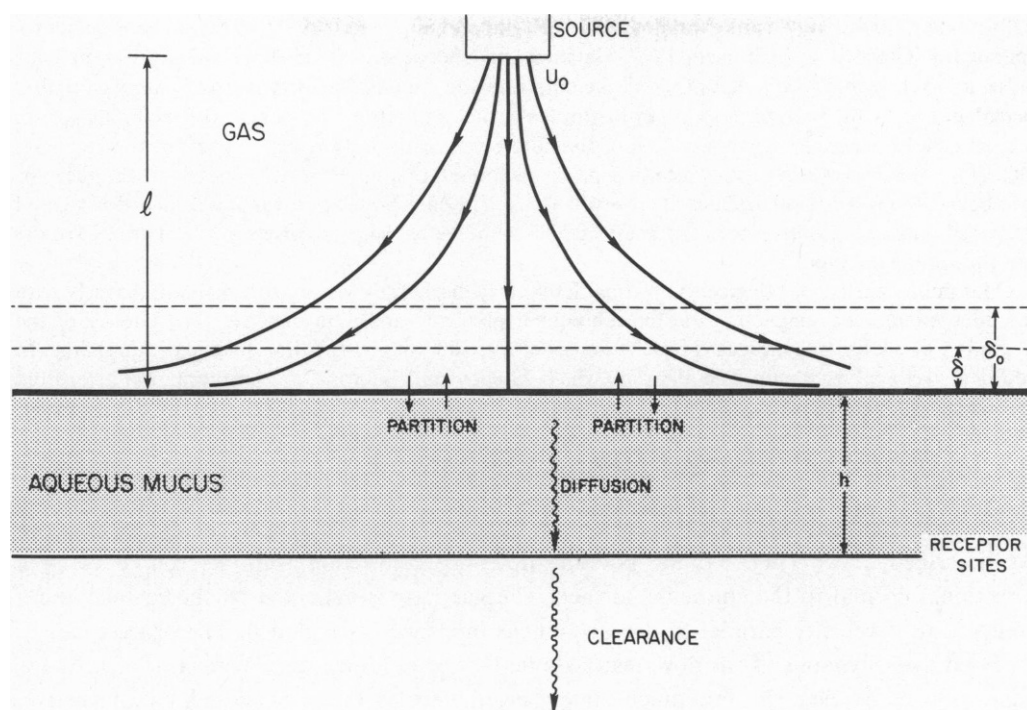


FIGURE 1 Diagrammatic representation of the delivery of an odorant to the olfactory receptor sites. The odorant, diluted in a gas, emerges from a source nozzle located at a distance l from the surface of the mucus. The stream is deflected by the interface. The odorant diffuses through the distance h through the mucus to the locus of the effective receptor sites, where it elicits an excitatory response when the concentration reaches threshold.

interface. An analysis of data published on responses evoked by other odorants further supports the conclusion that odor receptor sites lie deeply in the mucus layer at the level of the apical dendrite of the neuron, the proximal ciliary segments, and the microvilli of the sustentacular cells.

MATERIALS AND METHODS

Extracellular unitary recordings were obtained from the olfactory epithelium of the tiger salamander, *Ambystoma tigrinum*. Surgical and preparative techniques have already been described (Kauer, 1974; Getchell, 1977b; Getchell and Shepherd, 1978a). The receptor cells are quite small and fragile. This presents severe difficulties in efforts to obtain intracellular recordings from identified cells (Getchell, 1977b; Suzuki, 1977). Therefore, platinum black filled micropipettes were fabricated as previously described (Getchell, 1973). They had plated tip diameters of $\sim 5 \mu\text{m}$ and resistances of $\sim 0.25 \text{ M}\Omega$. The use of these electrodes gave optimal signal to noise ratios and ensured long enough recording times (up to 2 h) to enable us to investigate the unit response properties to changing odor concentration. Specific criteria for assessing the possible effects of electrode impingement on spike conformation and response characteristics were as described previously (Getchell, 1973).

The use of quantitative methods for odor delivery are necessary for determining the parameters of the excitatory discharges. We used odor pulses which were controlled and monitored. The techniques have been developed over recent years and applied to the analysis of responses recorded from the olfactory

bulb (Kauer, 1974; Kauer and Moulton, 1974; Kauer and Shepherd, 1975; 1977) and the olfactory epithelium (Getchell and Getchell, 1977; Getchell and Shepherd, 1978a; 1978b). Basically, the odor pulse is mixed with 5% CO₂/95% O₂ and the output of the stimulus delivery nozzle is sampled with a Beckman LB-2 CO₂ analyzer (Beckman Instruments, Inc., Fullerton, Calif.) near the recording site on the epithelial surface. Proper orientation of the nozzle was critical. Temporal aspects of the response, including latency, have been shown to depend on the orientation of the nozzle relative to the receptor unit being monitored (Getchell and Shepherd, 1978a). The odor pulse approximates a square wave, and is treated as though it were one in our analysis. The rounding of the pulse onsets in Fig. 3 are due to the rise time of the monitor.

Odor pulses were of varying concentration. Excitatory discharges were recorded simultaneously with the odor monitor on magnetic tape for subsequent playback and data analysis. The latency of the excitatory discharge was measured from the onset of the odor pulse to the first spike in the discharge. In addition, we analyzed published data in which latency and odorant concentration were reported (Shibuya, 1969; Holley et al., 1974).

THEORETICAL ANALYSIS AND RESULTS

Rate Limiting Processes

As described above (Fig. 1), the odorant flows by convection from its source along a directional normal to the epithelial surface. The gas flow is deflected by the surface and a component of velocity parallel to the gas/mucus interface is developed. The process can be modeled hydrodynamically as flow near a stagnation point (Jones and Watson, 1963). As the odorant streams over the gas/mucus interface, molecules are transported by convective-diffusion toward it. The velocity of the gas effectively determines the thickness of the hydrodynamic boundary layer, δ_0 . The diffusion boundary layer thickness δ , can be related precisely to the hydrodynamic boundary layer thickness, but for our purposes the following approximation based upon a scaling of the convective-diffusion equations will suffice:

$$\delta = 0.5\delta_0 \left(\frac{D_g}{\nu} \right)^{1/3}. \quad (1)$$

Here D_g is the diffusion coefficient of the odorant in the gas phase and ν is its kinematic viscosity (cf. Levich, 1962). From Eq. 1 we can arrive at an estimate of the distance the gaseous odorant must diffuse before encountering the gas/mucus interface. With D_g , we can then estimate the time required for this phase of stimulation and readily show that it is far too short to account for the latency of the neural response.

From the solution to the Navier-Stokes equation for stagnation flow, the hydrodynamic boundary layer thickness is estimated as:

$$\delta_0 \approx 2.4 \sqrt{\frac{\nu\lambda}{U_0}}, \quad (2)$$

where λ is the radius of the area being stimulated and U_0 is the velocity of the gas stream at the source. Strictly speaking, Eq. 2 locates the plane above the interface for which the parallel component of the velocity has 99% of the limiting value approached asymptotically with distance from the surface. The time, t , required for diffusion through a distance δ is of the order of δ^2/D_g . Using Eq. 1 and 2 we obtain:

$$t \approx \frac{1.44\lambda}{U_0} \left(\frac{D_g}{\nu} \right)^{-1/3}. \quad (3)$$

For gases, D_g/v is of the order of unity, $\lambda = 0.25$ cm and U_0 is the velocity corresponding to a volumetric flow rate in ml/s through an orifice of 0.1 cm diameter. For a typical flow rate of 2 ml/s, t is found to be ~ 1.4 ms. The observed latencies range from 45 to 2,000 ms, so diffusion to the gas/mucus interface is far too rapid to be rate limiting. Similarly, partitioning just across the interface can be expected to be fast compared to diffusion in the aqueous layer. This then suggests diffusion in the aqueous mucus as the likely process capable of accounting for the latencies observed. This is reasonable since diffusion coefficients in the aqueous phase are about four orders of magnitude smaller than in the gas phase. It is unlikely that the transduction process itself is responsible for the long latencies reported, since most sensory receptors produce a neural response within a few milliseconds when presented with a suprathreshold stimulus (Fuortes, 1971).

Diffusion in the Aqueous Phase

It is generally acknowledged that odorants must diffuse to some extent through the superficial layer of mucus before encountering the receptor cells (Ottoson, 1971). The fate of the odorant after reception may depend largely on its chemical structure. Hornung and Mozell (1977) have shown that butanol may be removed from the bullfrog olfactory mucosa by three processes: (a) transport back from mucus to air, (b) transport with mucus into the oral cavity, and (c) clearance into the general circulation. In addition, Bannister (1974) and Getchell and Getchell (1977) have suggested the possibility of chemical alteration in the mucus or at the receptor site. From a theoretical standpoint the mode of clearance is important because it specifies a boundary condition on the diffusion process. Condition *a* suggests a reflecting surface some finite distance beyond the receptor loci; conditions *b* and *c* suggest diffusion into a semi-infinite domain. Chemical removal by enzymic reactions implies a gradient of odorant just supported by adsorption or a chemical rate process governing removal. Conditions *a*, *b*, and *c* are similar in that they imply a vanishing concentration gradient at the boundary. They differ only in that *a* requires a finite location close to the receptor plane whereas *b* and *c* imply clearance at some remote location.

For purposes of developing the model we shall treat herein only the semi-infinite case. It is the simplest mathematically and does not require the additional parameters inherent in the general reflecting surface or the chemical clearance models. However, we have completed calculations on a modified reflecting surface model assuming the reflecting surface and the receptor plane to be identical. In this case we calculated the receptor depth to be essentially the same as in the semi-infinite model. In the latter case, the governing equations are:

$$\frac{\partial c^*}{\partial t} = D \frac{\partial^2 c^*}{\partial x^2}, \quad (4)$$

$$c^*(0, t) = c_0^*, \quad (5)$$

$$\left. \frac{\partial c^*}{\partial x} \right|_{\infty} = 0, \quad (6)$$

$$c^*(x, 0) = 0. \quad (7)$$

Here, $c^*(x, t)$ is the concentration of odorant inside the aqueous layer, D is its diffusion coefficient which we assume is equal to its value in water and constant over the semi-infinite

domain, x is the coordinate normal to the mucus plane, t is time, and c_0^* is the odorant concentration just inside the gas/mucus interface ($x = 0$). The solution is simply:

$$c^*(x, t) = c_0^* \operatorname{erfc} \left(\frac{x}{2\sqrt{Dt}} \right), \tag{8}$$

where erfc is the error function complement (Carslaw and Jaeger, 1959). The aqueous concentration c_0^* is related to the stimulating concentration c_0 in the gas phase through a partition coefficient, K , as $c_0 = Kc_0^*$. It is desirable to express Eq. 8 in terms of the applied stimulus concentration, viz:

$$c(x, t) = c_0 \operatorname{erfc} \left(\frac{x}{2\sqrt{Dt}} \right), \tag{9}$$

where $c(x, t) = Kc^*(x, t)$. This quantity can be viewed as a normalized local concentration of odorant.

Fig. 2 shows concentration time courses at a fixed depth of 40 μm below the gas/mucus

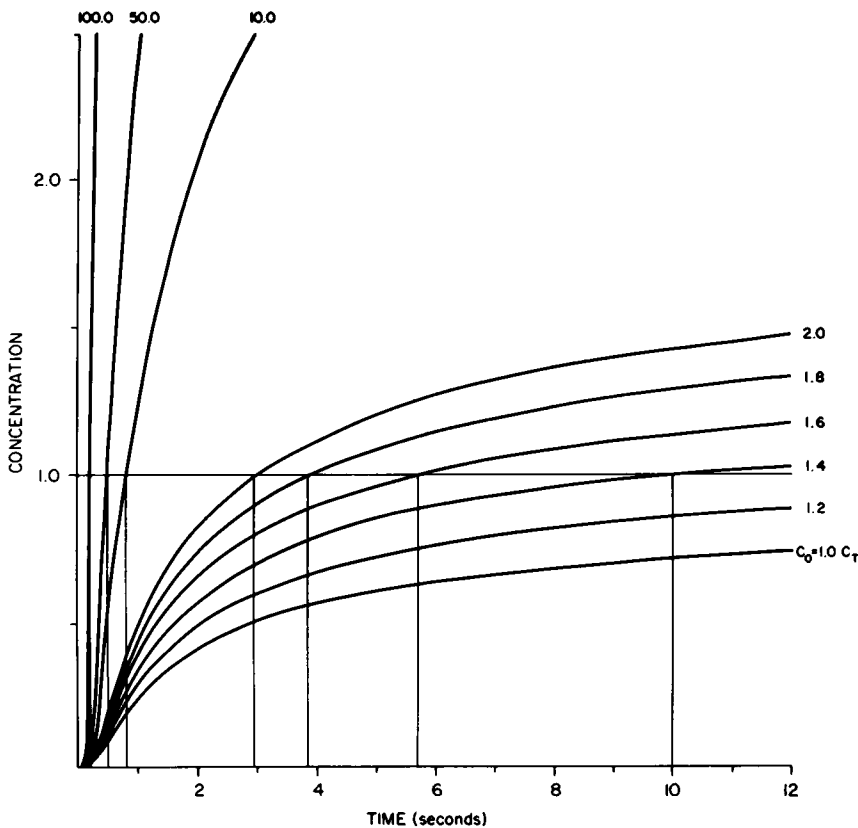


FIGURE 2 The time course of concentration variation at a given depth in the mucus with varying gas phase concentrations of odorant. The concentrations are expressed relative to the threshold, c_T . The vertical lines show the latencies that occur when different gas phase concentrations are used.

boundary for a variety of stimulus concentrations. The line of constant concentration, c_T , represents the apparent threshold concentration above which the receptors are excited by that odor. The absolute threshold, c_T^* , is, of course, equal to c_T/K . Analysis shows that if c_0 equals c_T , threshold cannot be attained in a finite time. If c_0 is raised, the time required for $c(x,t)$ to reach c_T shortens. The observed latency, therefore, is determined by the apparent threshold concentration, the diffusion coefficient of the odorant and the depth of the receptor site in the mucus. If the latency is determined for a series of stimulating concentrations of a given odorant, it is possible to predict the apparent threshold concentration and the probable location below the gas/mucus interface at which the initial stage of transduction occurs.

Results for CO₂ and Safrole Stimulation

Systematic changes occur in the parameters of an excitatory discharge recorded from an olfactory receptor neuron in response to changing odorant concentration. An example of these changes is shown in Fig. 3. Each trace shown displays the spike potentials (upper trace) and the simultaneously recorded output of the CO₂ monitor (lower trace). The low level background of spontaneous activity is shown. It was estimated to be ~20 spikes/min from recordings of long prestimulus intervals. The responses to increasing concentration are shown in traces *b-g*. Unambiguous excitatory responses evoked by increasing stimulus concentrations are shown in traces *c-g*. At the lowest concentration the response consists of a simple tonic

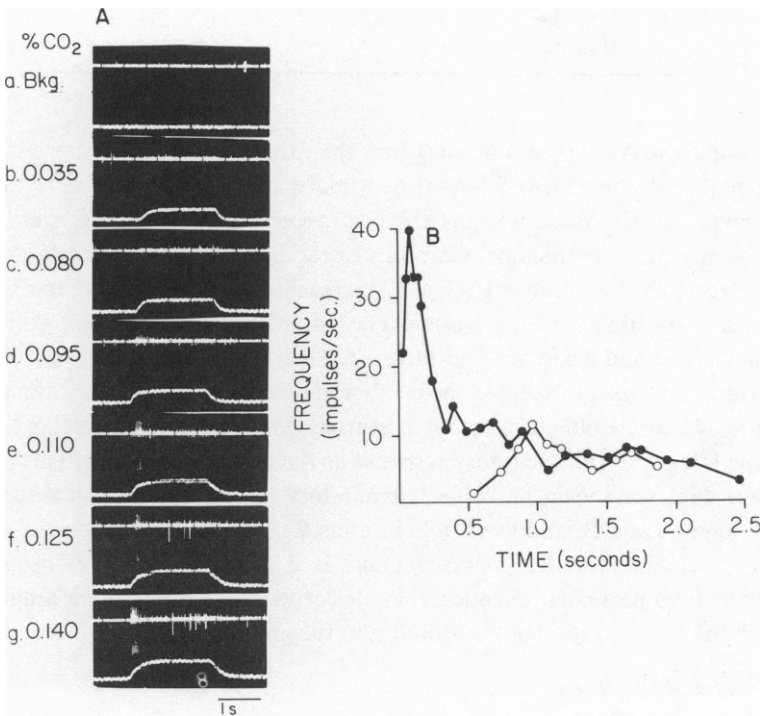


FIGURE 3 (A) Unitary recordings (top trace) from olfactory receptor neurons are shown simultaneously with the pulse monitor (lower trace). Representative background activity is shown in *a*; *b-g* show responses evoked by increasing concentrations of CO₂ in carrier air. (B) The instantaneous frequencies of the responses to near threshold (open circles) and high (filled circles) concentrations of CO₂.

TABLE I
VARIATION OF LATENCY WITH GAS PHASE ODORANT
CONCENTRATION IN OLFACTORY RECEPTORS

Stimulus and concentration	Latency	
	Unit A	Unit B
	(ms)	(ms)
CO ₂		
0.035%	No response	No response
0.065%	535	No response
0.080%	170	No response
0.095%	105	1,435
0.110%	80	1,090
0.125%	65	1,015
0.140%	45	965
	Unit C	Unit D
Safrole		
18 nM	No response	No response
36 nM	1,977	No response
44 nM	1,379	425
51 nM	1,034	325
58 nM	885	315
65 nM	660	275
73 nM	637	235
140 nM	517	150

discharge of impulses. At higher concentrations the excitatory responses consist of an initial high frequency phasic component followed by a more nearly constant rate of discharge, the tonic component. At all concentrations there is a precise temporal register between the excitatory response and the monitor. Careful inspection of the traces shows that there is a systematic decrease in the onset latency with increasing concentration, from 535 ms at the lowest to 45 ms at the highest concentration. The instantaneous frequencies of the discharges elicited by the highest and lowest concentrations are displayed in Fig. 3 B.

Intensity-response functions have been thoroughly studied in excitatory discharges recorded from ~45 single olfactory receptor neurons. We consistently observed a systematic decrease in the latency of the excitatory response as the odorant concentration was increased. Representative data are shown in Table I. Excitatory discharges were evoked by CO₂ and carrier air in units A and B, and by safrole in units C and D. The latencies of the responses vary inversely with the stimulus concentrations. It is clear that there is no characteristic response latency for a particular chemical stimulus or for a given cell, but a range of latencies that vary with the concentration of the stimulus in the gas phase.

Analysis of the Data

We denote the apparent threshold concentration as:

$$c(h, t_L) = c_T, \quad (10)$$

where the left hand side of Eq. 10 is the apparent concentration at the receptor depth, h , and t_L

is the observed latency. With Eqs. 9 and 10 we obtain

$$t_L = \frac{h^2}{4D \left[\operatorname{erfc}^{-1} \left(\frac{c_T}{c_0} \right) \right]^2}, \quad (11)$$

where erfc^{-1} denotes the inverse error function complement. In Eq. 11 we can regard h and c_T as parameters which, when properly determined, provide an accurate prediction of the latency at any stimulating concentration. These parameters were determined using a nonlinear least squares criterion and employing a modified Gauss-Newton procedure (Metzler et al., 1974).

In the case of CO_2 stimulation we find the following estimates of threshold and receptor depth: $c_T = 0.05\%$ and 0.03% ; $h = 11$ and $64 \mu\text{m}$. For safrole the thresholds were 21 and 15 nM, the receptor depths were 30 and $21 \mu\text{m}$. Fig. 4 shows the latency normalized with the characteristic time h^2/D as a function of the normalized stimulating concentration for CO_2 and safrole. The points are plotted on the same graph using $D = 1.45 \times 10^{-5} \text{ cm}^2/\text{s}$ for CO_2 (Jost, 1960) and the least squares estimates of c_T and h to scale the individual values. A measured value of D for safrole is unavailable. Tucker (1963) reported a value of $6 \times 10^{-6} \text{ cm}^2/\text{s}$ as the value of D for amyl acetate. Since the molecular weights of safrole and amyl acetate differ by $<20\%$, the diffusion coefficients for the two compounds should be similar. Therefore, we have used $6 \times 10^{-6} \text{ cm}^2/\text{s}$ as the value of D for safrole. As can be seen in Fig. 4,

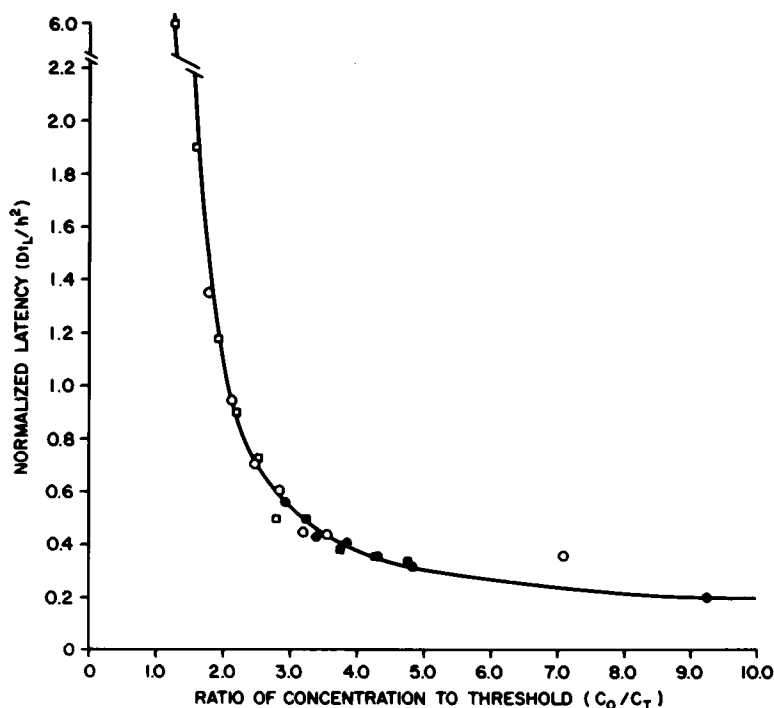


FIGURE 4 Normalized latency is plotted as a function of relative concentration of odorant in the gas phase for two odorants, safrole (circles) and CO_2 (squares). The open and filled symbols represent different units. The line is a theoretical curve.

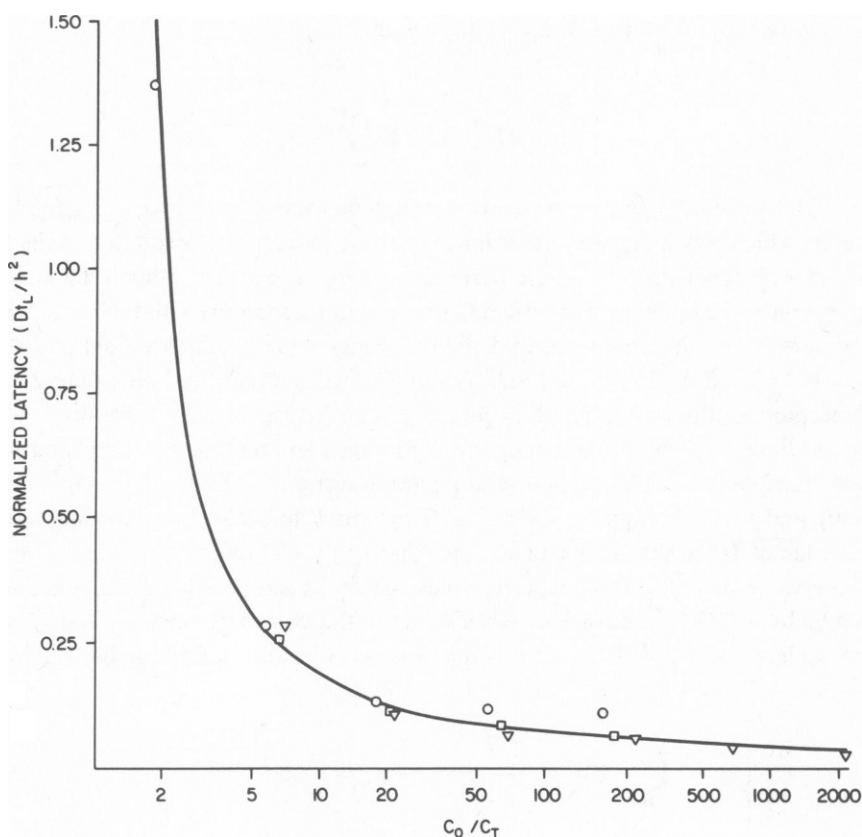


FIGURE 5 Normalized latency is plotted against relative concentration for three units, represented by the three symbols, responding to amyl acetate. The data are taken from Shibuya (1969). The line is a theoretical curve.

the diffusion model with two parameters gives an accurate representation of the dependency of latency on concentration for both compounds.

As a test of the generality of the model we have applied it to data in the published literature. Shibuya's (1969) results with amyl acetate in three olfactory units of the tortoise are shown in Fig. 5. The latencies of the excitatory responses were measured from the onsets of the simultaneously recorded electroolfactograms. Data of Holley et al. (1974) for amyl acetate stimulation in the frog are shown in Fig. 6. In each case, Tucker's (1963) value of 6×10^{-6} cm^2/s was used for the diffusion coefficient. The diffusion model satisfactorily accounts for the data. Table II includes the parameters estimated from these data. For each of the units reported by Shibuya (1969) the receptor locus is calculated to be ~ 50 μm below the gas/mucus interface. The apparent thresholds are the same for two of these units, while the third is more sensitive by tenfold. The data of Holley et al. (1974) give an apparent depth of 97 μm . The latencies in that study were measured from the time of opening of a valve controlling odorant flow. Thus, an unknown length of time, needed to clear a dead space between the valve and the odor delivery nozzle, is included in the measured latencies.

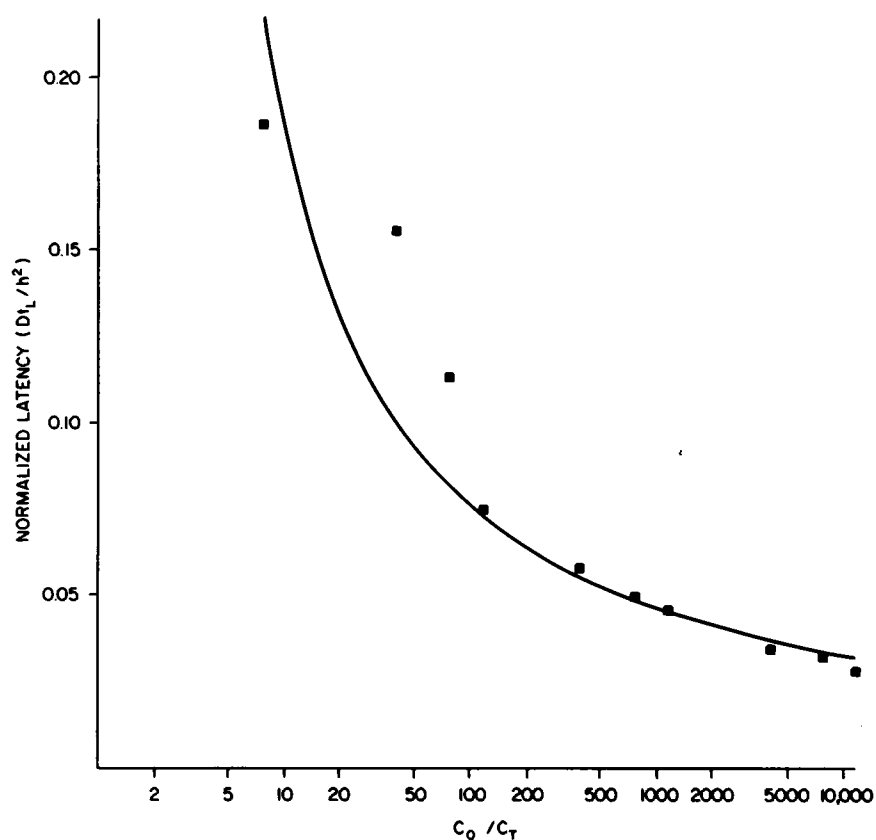


FIGURE 6 Normalized latency is plotted against relative concentration for response of a unit to amyl acetate. The data are taken from Holley et al. (1974). The line is a theoretical curve.

TABLE II
PARAMETERS FOR OLFACTORY RECEPTOR UNITS CALCULATED FROM THE MODEL
DESCRIBED IN THIS PAPER

Stimulus	Threshold (c_T)*	Receptor depth (h)*	Source of data
CO ₂	0.050%	11 μ m	This paper
	0.030%	64 μ m	This paper
Safrole	21 nM	30 μ m	This paper
	15 nM	21 μ m	This paper
Amyl acetate	$4.9 \times 10^{-3}\ddagger$	48 μ m	Shibuya (1969)
	$5.5 \times 10^{-3}\ddagger$	48 μ m	Shibuya (1969)
	$4.6 \times 10^{-4}\ddagger$	46 μ m	Shibuya (1969)
Amyl acetate	4.3 nM	97 μ m	Holley et al. (1974)

*Standard errors on c_T and h do not exceed 7% of the means.

\ddagger Concentration expressed as fraction of saturated vapor.

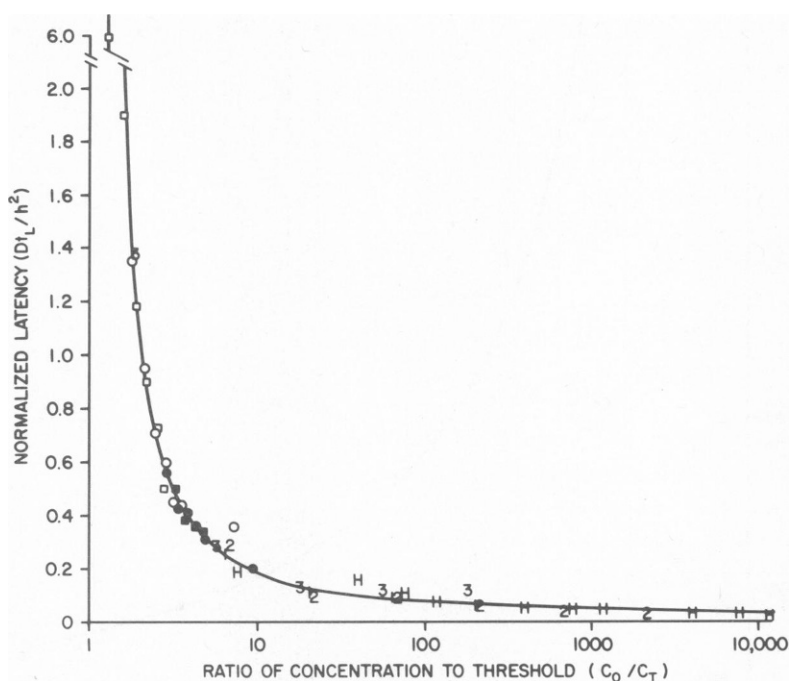


FIGURE 7 A composite of the data presented in Fig. 4-6, normalized for the various apparent receptor depths and thresholds (see Table II). The symbols are: circles, our safrole units; squares, our CO₂ units; 1, 2, and 3, the units reported in Shibuya (1969); H, the unit reported in Holley et al. (1974).

Therefore, 97 μm represents an upper limit to the distance from the mucus surface to the receptor sites.

Fig. 7 is a composite of the data analysis for all the units discussed. Each latency has been normalized by the characteristic time h^2/D for each substance and neural unit, and each concentration by the apparent threshold. There is adequate agreement with the theoretical curve over the full range of latencies and concentrations.

DISCUSSION

We have modeled the latencies recorded from olfactory receptor neurons as being primarily due to the time required for diffusion of stimulus molecules to reach threshold concentration at the receptor depth. We have treated the stimulus presentation as though it was a true square wave. This simplifies the treatment while introducing only small errors. Analysis of our data and published data from other laboratories gives excellent agreement with the theoretical model. In each case the depth at which the receptor molecules lie is calculated to be 10-70 μm . This is virtually independent of whether one assumes that the apical surface of the olfactory epithelium presents no barrier to diffusion (the model used here) or that it presents a total barrier to the diffusion of the odorant. It is interesting that the calculated threshold concentrations for CO₂ were similar in both of the units that responded to it, that the thresholds for safrole were nearly the same in the two safrole sensitive units, and that the

thresholds for amyl acetate were the same in two of the three units reported by Shibuya (1969).

There are a number of other possible explanations for the long latencies recorded from receptor neurons, which we believe to be less than satisfactory. For example, one might imagine that transduction itself is a slow process. Bostock (1974) found that he could fit the time course of the electroolfactogram recorded from frogs to models very similar to the one employed here, but was forced to assume the existence of a 200-ms latency intrinsic to the receptor cells. We believe that the hypothesis that there is a long latency in olfactory transduction per se is unlikely to be correct. Other sensory receptors do not show this property. In addition, our experiments using CO₂ and safrole as stimuli provided data on odorants with diffusion constants differing by about twofold. Were the latencies due to inherently slow processes in the cells, these compounds would be expected to result in identical latencies. What was, in fact, observed is that the latencies are quite different, corresponding to the times needed to bring the concentrations to threshold at depths of 10–70 μ m.

Another possibility might be that interfacial kinetics are rate limiting. However, here a simple logarithmic relation between latency and threshold would be predicted. This would give anomalously low latencies at high odorant concentrations.

One might suppose that the receptor sites are very near the surface of the mucus but that carrier molecules exist which must combine with the odorants and which diffuse slowly because of their large size. This fails to account for the different latencies shown by receptor cells responding to different odorants. Finally, we can consider the possibility that the diffusion of the odorants from the mucus surface to the receptor sites does not follow a linear path but a tortuous one in which they must diffuse around closely packed cilia. First, the cilia do not appear to be so thick or so closely packed as to form a significant barrier or greatly lengthen the effective diffusion path. Second, most odorants are lipid soluble compounds, and membranous structures such as cilia would not be expected to block their diffusion. Finally, if one imagines that the distal parts of the cilia block access to the receptor sites, then one is assuming that the effective receptor sites are not exclusively on the distal parts of the cilia. This is precisely the conclusion reached by our analysis.

Where are the olfactory receptor sites? Electrophysiological data indicate that the current flow responsible for initiating the response originates on or near the apical surface of the receptor epithelium, perhaps in the proximal portion of the cilia (Ottoson, 1956; Byzov and Flerova, 1964; Getchell, 1977b). Bronshtein and Minor (1977) destroyed the olfactory cilia of frogs by treatment with a detergent. Although this abolished the electrophysiological response of the olfactory epithelium to butyl acetate, the responses recovered to 100% of the pretreatment values when the cilia were only regenerated to one-fourth of their original length. Goldberg et al. (1979) reported that the olfactory responses of mice can be blocked by topical application of antibody directed against what appear to be olfactory receptor sites. Since the molecular weight of the antibody is $\sim 160,000$, it is unlikely that the effect is on a site inside the cells or beyond the tight junctions between cell apices in the epithelium. Their study provides evidence that olfactory receptor sites are no deeper than the apical surfaces of the cells, which are ~ 30 – 50 μ m below the mucus surface. Thus, our data, taken with other lines of evidence, provide a strong argument that olfactory receptor sites are located on the cell membranes of the receptor cells in the region of the apical knob of the dendrite and the

proximal portion of the cilia. If molecular receptors exist on the membranes of the distal portions of the cilia, they are not effective in olfactory transduction; perhaps their small diameter results in high internal resistance to current flow.

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